SURVIVAL ANALYSIS OF COMMERCIAL STOCK *PINUS RADIATA* (D.DON) OUTPLANTED IN A PITCH CANKER INFECTED STAND

***a part of the IMPACT study lead by ENSIS and Dr. Colin Matheson**

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ABSTRACT

Survival Analysis of Commercial Stock *Pinus radiata* (D.Don) Outplanted in a Pitch Canker Infected Stand

Nathan A. Smith

Fusarium circinatum (Nirenberg & O'Donnell) or pitch canker, has the potential to have a tremendous impact on the economies which depend on the harvest and refined processing of *Pinus radiata* (D.Don) saw/"fiber" logs. This negative financial impact is compounded with environmental implications. These reasons are why Chile, New Zealand, and Australia, the principal producers of exotic *Pinus radiata* have entered into a coalition under ENSIS with the title of the IMPACT project (Balocchi et al., 1999). The field trial associated with the IMPACT project was located at Swanton Pacific Ranch; Cal Poly San Luis Obispo's Ranch in Davenport, California. Soil moisture and lesion lengths, non-native origin, both macro (national) and micro (tree breeder) were correlated with survival. Due to a natural drainage and a wet winter, high soil moisture had a negative impact on survival in the establishment phase. Lesion length also correlated with survival, the longer the lesion length found in Phase I, the more likely the stock would have a poor survival rate. It was also theorized by observations made during propagation, by Annie Mix and Dr. DetLev Vogler that the seed's origins and sub origins affected their survival during propagation and could potentially affect the survivorship in the field trial. Each of the participating nations employs various seed collection, storage and pollination methods which could explain some differences in survivorship. The evaluation that resulted from this research based on Origin and Sub origin supported that hypothesis. With all of these inherent factors, before resistance conclusions can be made, the overall validity of the comparison must be determined.

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INTRODUCTION

Pinus radiata (D.Don) is a unique pine. It is native in three restricted zones of California and two islands off of the coast of Mexico. There are 4,500 hectares of *Pinus radiata* in North America where it is native, (reported by Gordon et al. 2001) and roughly 3,763,000 hectares (Table 1) of Monterey Pine grown exotically in plantation form (Balocchi et al., 1999). Chile, New Zealand, and Australia are the principal nations which have very large plantations of Monterey Pine; consequently, they have entered into a coalition under the IMPACT project title to prepare for the fungus known as *Fusarium circinatum* (Nirenberg and O'Donnel) which causes pitch canker. This fungus could devastate their commercial plantations as it already has in California's native stands and ornamental plantings.

Table 1. Extent of *Pinus radiata* plantations, domestic and abroad

Country	Plantation size in 000 ha
Chile	1380
New Zealand	1338
Australia	642
Spain	237
South Africa	66
Other Countries	100
Total non-native area	3763
Estimated Native population (California, Guadalupe and Cedros Islands)	5 to 6

*(adapted from Balocchi et al, 1998)

The IMPACT project also has other studies being conducted attempting to isolate a resistant seed stock. Such projects include a glass house study (controlled environment with artificial inoculation), a field trial (laying out the seedlings in a field using natural sources of inoculums), and a retest of identified resistant stock to double check the previous research.

The Glass House Inoculation began in April 2001. The seedlings were transferred to Pebble Beach Company greenhouse for inoculation and scoring of lesion length. These results identified natural response to *Fusarium circinatum* via lesion lengths. Each of the involved seed lots could then be compared to a field trial where the seedlings were raised in a plantation surrounded by a native stand of *Pinus radiata* which was already infected by *Fusarium circinatum*. As previously mentioned, these results at the end of four or more years of data collection could then be reanalyzed to confirm any findings of potentially resistant seed stock.

Pitch canker is a disease that is caused by a fungus known as *Fusarium circinatum* which creates a resinous canker and lesion at the point of infection. This lesion girdles the tree, cutting off distal flow of nutrients (Storer et al., 1997, Gordon et al., 2001). The overall problem of finding a "cure" to control *Fusarium circinatum* is too large to be attacked at once. Many things must be investigated to advance science to the point of comprehending the complete problem. Topics include the value of systemic induced resistance, and specifically, what rates of infection will encourage resistance (Bonello et al., 2001). However, identifying some naturally resistant *Pinus radiata* and evaluating them as possible substitutes for commercial species in the international market is a highly obtainable solution. This objective of identifying existing resistant commercial stock is the topic and the goal of the IMPACT project. Many of the commercial strains of *Pinus radiata* in the international market were outplanted at California Polytechnic State University's ranch near Davenport, California to detect natural environmental resistance to *Fusarium circinatum*. Swanton Ranch is bordered by and contains a portion of the southern end of the native Aňo Nuevo Stand of *Pinus radiata.* (Devey et al., 1999) The goal of isolating *Pinus radiata* from Chile, Australia and New Zealand (the participants), which are resistant and already proven performers, is a cost-effective and easily-implemented resistant regeneration regime, as they already have the facilities and controlled cross seed to sow. To effectively identify stock which has significantly higher resistance and survival in a field setting, the data will be analyzed in SAS' multivariate repeated measures analysis, examining the data in both liberal and conservative evaluations allowing the reader to decide the appropriate management solution. There are enormous possible management implications associated with a possible infestation of *Fusarium circinatum* to the plantation owners.

LITERATURE REVIEW

Identification and Effects of Pitch Canker

The fungus can be identified primarily by the occurrence of lesions or cankers on the branch or main stem which occur at branch nodes. These cankers "ooze" excessive amounts of pitch hence the name pitch canker (Gordon et al., 2001).

Fusarium circinatum or pitch canker is a fungus that has a girdling effect on the tree, meaning that it stops nutrient flow and kills all tissue distal to the infection point. This is not the end for all trees. Many trees, as long as they are not overwhelmed with infected branches, can just "slough it off." Sloughing it off is a term describing the tree's ability to self-prune or drop the infected branches off the tree. If, however, the tree is overwhelmed with multiple infected limbs, it cannot keep up with the required photosynthetic rate for survival. Also, if the tree has a bole infection, meaning that it has a canker on the main-stem which girdles it, the entire tree will probably die and be subject to ensuing rot (Owen et al., 1998). If pitch canker reached any of the countries that depend on *Pinus radiata*, and did infect some of their plantations, many, if not all, of the trees, would suffer growth inhibition if not death. This possibility has been realized by three of the nations (Chile, New Zealand and Australia) so they have begun researching resistance and analyzing data from some researchers.

In an experimental plot in Central California, the effects of pitch canker were followed for more than four years to determine the effect of the disease on Monterey Pine. 87% of the pines that were removed had pitch canker (not necessarily fatal level) and less than 3% of the trees remained pitch canker free (Storer et al., 1999).

The implication is that there is some near-complete resistance to pitch canker in native stands, however little it is. As little resistance as the native stands have, the large genetic pool in its native range will aid in the future development of resistant pine, and Monterey pine will continue to persevere. However, there is considerable genetic similarity of the trees in the plantations of Chile, New Zealand and Australia, resulting from various selective breedings for performance, not resistance. These plantations will most likely not have the same result as the experimental plot in the native stand.

History of Pitch Canker

Pitch canker is the term referring to the tree's response to a fungus known as *Fusarium circinatum* formerly *Fusarium subglutinans* (Wollenweb. & Reinking 1983) which has been identified in North America since 1946. It was believed to be a minor pine disease of Southeastern United States (Wikler et al., 2000, Correll et al., 1991). Not certain as to its global implication, little was done in the area of quarantine or active management because it was not considered to be a major threat to the timber industry. Tracking its mode of travel, and where it originated, is difficult because no one is definitively certain as to where it originated. Information learned about a *Fusarium circinatum* population in Mexico, which has proven to be more genetically diverse than in California and Florida. This implies that it existed in Mexico longer than in the Southern United States, as previously assumed (Gordon et al., 2001). The previous assumption was that the first-time pitch canker was reported was in the Southern United States in 1946, and its origin was determined to be in Haitian pine (Hepting et al., 1946). The "blame" for the associated spread of pitch canker in the

United States is hard to determine, as we are not certain as to where the infected material's origin is, or even what material catered to its spread. However, we are certain that it is currently in California (Wikler et al., 2003). In 1986, the first case in California was identified in the Santa Cruz/Bay Area of Northern California.

In response to the first identification and rapid spread of *Fusarium circinatum* in Northern/Central California, an interagency working group of local county governments evaluated the infected population determining the disease had been present in the region for a number of years before its formal recognition (Owen et al., 1998). Between the first report in 1986 and 1991, it had spread all throughout the native stands and ornamental stock in California.

Prior to pitch canker's spread into California's native and ornamental stands of *Pinus radiata,* it was voracious in many Southeastern seed plantations of *Pinus elliottii* (Engelm.), slash pine and *Pinus taeda* (L.), Loblolly pine. In 1974 it hit "epidemic" proportions in those plantations and received national notoriety (Dwinell et al., 1985, Dwinell 1998). Not long after, it headed west and was officially identified in California, in 1986. Since 1986 *Fusarium circinatum* has been reported to occur in Japan, Haiti, in some container and hedge stock in nurseries in South Africa, Spain (Dwinell, 1998, Viljoen et al., 1997), and Chile (Wingfield et al., 1998).

Interestingly, though *Fusarium circinatum* is showing prevalence in container and greenhouse populations of Monterey pine in South Africa and Chile, it has yet to be identified in adult populations in those countries. Possibly a vector or injury causing agent, improper climate, or other missing link in the requirements for infection of adult trees is missing (Dwinell, 1998). In 1997 the first incident of pitch canker was reported in a *Pinus radiata* commercially dependent country, the Basque area in Northern Spain. This incidence was isolated to a few bare root nurseries, and was either properly controlled or the climate was not conducive to continued infection. In 1998 there were no reoccurrences in the nurseries or in the mature plantations (Dwinell, 1998). As mentioned above, Spain experienced a one-time occurrence of pitch canker infecting a greenhouse population. Contrastingly, South Africa and Chile are experiencing a long lasting occurrence of pitch canker in the nurseries' bare root stock and hedge material (Viljoen et al., 1997, Wingfield et al., 1998), with no existence of the fungus outside of the nursery.

Pitch canker's existence in the United States is neither contested nor restricted to nurseries. Monterey pine's current established geography within the United States, spans from Virginia south to most all of Florida and west to eastern Texas, and predominantly, central California (Dwinell 1998, Dwinell et al., 1985, Correll et al., 1991). The current "widespread" establishment of pitch canker combined with the globalization of trade and commerce between all of the *Pinus radiata* dependent countries provides the means for the fungus to infect all of the countries.

Importance of *Pinus radiata* to the World

Monterey pine or *Pinus radiata* is the most extensively planted exotic conifer in the world. The expansive nature of *Pinus radiata* aids in the ability of pitch canker to infect foreign plantations. Considering the very real possibility of pitch canker spreading across all obstacles to infect vast plantations, it is important to understand the overall importance of Monterey pine to the world. Non-natively, there are approximately 3.8 million hectares planted worldwide. The five primary countries are

Chile, New Zealand, Australia, Spain and South Africa. Each of these nations has varying sizes of plantations, but Chile and New Zealand have the most expansive plantations – more than 1,300,000 hectares each (Balocchi et al., 1999). The commercial use is quite expansive and is even more vivid when contrasted with the statistics of its native range. Monterey pine populations within its native range: California's Central Coast (United States), specifically Año Nuevo, Cambria and Monterey, and Mexico's Cedros and Guadalupe Islands, constitute little more than 4,500 hectares (Gordon et al., 2001).

The economic value of Monterey pine to Australia, New Zealand, Chile and others, does not solely lie in the production of logs, but in the refined products as well (Anon. 1988, Burdon 1992). Primarily, the plantations serve a renewable source of saw timber, timber that can be used as structural lumber in home construction, and any other construction that requires dimensional lumber. The trees are also used as a source of fiber for paper, particle board, oriented strand board, panels and other industrial products (Balocchi et al., 1999, Anon. 1988, Burdon 1992). In addition to the obvious economic benefit as a source of timber, the Monterey pine plantations are effectively diverting the harvest pressure off of the rare native trees, which cannot grow as quickly or as efficiently as the stands of planted Monterey pine. Specifically, these plantations produce nearly 50 million cubic meters per year (Table 2) and fuel expansive economic developments in Australia, Chile, New Zealand, Spain and South Africa (Balocchi et al., 1999).

Table 2. Annual wood production of *Pinus radiata*

Country	Actual wood production (000 m^3/yr)	Projected (yr. 2036) Wood Production (000 m^3/yr)
Chile	18,548	45,000
New Zealand	17,000	43,000
Australia	10,400	12,000
Spain	2,000	3,000
South Africa	486	1,000
Total non-native area	48,434	104,000

*(adapted from Balocchi et al, 1999)

Pinus radiata is not native to any of the nations which now depend on it as a commercial species. Knowing where these trees originated may be critical for understanding their future. No one is certain where the first collected seed from California originated, but we do know that its genes have contributed to the development of some exceptional commercial stock. This stock has been developed into vast plantations of successfully performing fiber producers. Many research and development firms have returned to native stands of Monterey pine multiple times over the years not only to broaden their genetic pool but to identify more impressive genetics. This effort to broaden the genetics is specifically observed in Chile's 1991 campaign by CAMCORE or the Central America and Mexico Coniferous Resources Cooperative to broaden its genetic base by going to the various native locations of Monterey pine to select 90 mother trees (as reported by Balocchi et al., 1999). The value of *Pinus radiata* as a timber source in foreign countries is extensive. Similarly, the impact that pitch canker would have on the commercial producing countries could be as extensive.

In 1985, *Pinus radiata* it was estimated to total 50 million standing trees, in both its native range and as ornamental trees in California (Gordon et al., 2001).

Within California, Monterey pines were widely planted along highway corridors (such as Highways 1 and 101) because of their ability to survive in California with fast growth rates. Their rapid growth is valued because it quickly leads to a rapid visual impediment and sound obstruction to screen the highways from adjacent properties (Gordon et al., 2001). The predominance of Monterey pine along highways may have aided in its local (California) demise. The nature of how *Fusarium circinatum* can remain on a beetle or in the wood, which is transported as firewood from town to town, allows it to be easily spread from region to region, from native stands to ornamental trees and back. Monterey pines, with their incredible growth rate, precipitate great value as an ornamental tree. The implied value as a rare, beautiful resource in its native range was an Achilles-heal from the combined threats of residential development and pitch canker, *Fusarium circinatum* (Owen et al., 1998). In addition, all of the foreign countries depend on the genetic diversity as a bank of genetic resources for improving their commercial plantations, which has and is continuing to shrink due to the pitch canker onslaught (Owen et al., 1998). This biological nemesis of Monterey pine has impacted it in its native range, where genetic diversity is high. Imagine what could happen in the narrow genetic pools of Chile, Australia and New Zealand.

Potential impacts of pitch canker

When pitch canker reaches mature plantations in New Zealand, Australia, Chile and the other nations which have smaller plantations of Monterey pine, like South Africa and Spain, it will have a catastrophic proportional impact on each of the respective economies, or will it? It has been argued that even if it were to reach these countries there would be little impact as seen in the "infection" of Spain, Chile and South Africa. There are many requirements for *Fusarium circinatum* to be an effective pathogen, two being that it needs the appropriate climate, and that it needs to have a suitable pathway to infect the trees. The assumption is that if it does so well here in California, then we should be able to compare the climatic conditions of California and the climatic conditions in the foreign countries (Table 3) to determine if it would be conducive to further infection.

Table 3. International climatic comparison table.

(note: Numbers in parentheses (#) or red are below California's average, and all others are above California's average; adapted from Balocchi et al. 1999)

Country	Annual rainfall (mm)	Avg. compared to California (surplus in mm)	Annual temp (^{0}C)	Avg. compared to California	Coldest month (^{0}C)	Avg. compared to California	Hottest month (^{0}C)	Avg. compared to California	Absolute minimum (^{0}C)	Avg. compared to California
USA, California	420-700	None	13-15	None	10-11	none	16-18	None	-7	none
Australia	650-1300	415	11-14	(1.5)	0.4-6	(7.48)	24-30	10	-10	(3)
Chile	450-2500	915	11-15	(1)	7-12	(1)	16-21	1.5	-10	(3)
New Zealand	700-1500	540	8-13	(3.5)	3-6	(6)	13-17	(2)	-10	(3)
South Africa	900-1000	390	9-12	(3.5)	9-11	(0.5)	10-13	(5.5)	-3	4

Prior to evaluating the data collected in this Phase of the IMPACT project a critical question must be answered. Can *Fusarium circinatum* survive and potentially thrive in Australia, New Zealand and Chile? The evidence reported by Gordon et.al. in *plant diseases* (2001) paints a picture in which the investigator admits that in laboratory settings, the existing population of *Fusarium circinatum* in California is capable of sexual reproduction. Interpreting this, *Fusarium circinatum* is heterothallic; it has mycelia of two dissimilar types, both of which must contribute for successful sexual reproduction. This has not appeared to date in native stands of

Monterey pine because the genetics of this population appear to be constrained; implying that most reproduction (initial surveys have estimated that probably all) is clonal and not encouraging of adaptation to the environment (Gordon et al., 2001, Wikler et al., 2000). The implication of this is that even if *Fusarium circinatum* does cross into the Monterey pine dependent countries, that specific strain may not perform well. However, if given the opportunity to sexually reproduce like it has in greenhouse studies in Japan (Gordon et al., 2001), it may expand outside of the nursery, a controlled environment, into plantations. This has the potential to be a catastrophic impacting variable in the international timber market.

Another argument supporting the idea that if *Fusarium circinatum* were to reach Australia, New Zealand, Chile, Spain, South Africa or other commercial *Pinus radiata* dependent countries it would not be a problem is that pitch canker requires appropriate vectors to impact the stand. This assumption is not only false on the simple pretense that pitch canker requires insect vectors, but that each environment does not have a suitable insect vector replacement to the typical insect pest of native Monterey pine, the Ips engraver beetle or *Ips paraconfusus* (Lanier). Although in California, there are several different insects that have proven to transport *Fusarium circinatum,* such as twig beetles, the cone beetle, and two other types of Ips beetles (Gordon et al., 2001). Each of the non-native locations within which Monterey pine have a tremendous ecological role has potential insect “vectors” to satisfy the vacant niche see Table 4.

Table 4. Primary Biological Damaging Agents.

Country	*Ryacionia buoliana*	Sirex noctilio	Hylaster spp.	Monkeys and Baboons	Kangaroos and possums	Rats and Mice
Australia		x	X		x	X
Chile	x		X			X
New Zealand		x	X			X
South Africa		x	X	x		X

*(adapted from Balocchi et al 1999)

Aside from the fact that the theory that pitch canker requires a vector, and that the other countries do not have suitable vectors, is null; studies have proven that the general wounding agent in the Southeastern United States population of *Fusarium circinatum* is wind, hail, and other naturally-occurring damage. The nature of plantations provides rubbing and pruning wounds (Gordon et al., 2001). The prevalence of naturally occurring wounding in the Monterey pine plantations provides potential risk from the occurrence of *Fusarium circinatum* in the plantation dependent countries.

The potential impact on Monterey pine has forced many of the nations to attempt quarantine and exclusion programs. Hosking et al. (1999) reported five "possible pathways of entry: seed, insects, timber and wood products, live plant material and used forest equipment and machinery." There are existing quarantine measures in effect in the respective countries to attempt to eliminate all risks associated with pest and disease incursions. The potential of these measures to successfully exclude pests and diseases is questionable as illustrated by Table 5. Specifically, Australia is at great risk because 670,000 cubic meters of sawn timber per annum are imported. Nearly 40% of that was *Pinus radiata* directly imported from New Zealand, and the rest was predominantly *Pseudotsuga menziesii* Franco from the United States and Canada (Anon., 2001) An important note is that the only non-pine which is susceptible to pitch canker is Douglas-fir (Gordon et al., 2006).

Table 5. Forest pathogen incursions and their likely mode of entry (1971-1998)

Pathogen	**First year of record (assumed infiltration of exclusion practices)**	***Main Host group***	**Mode of entry**	**Category of entry**
Melampsora medusae Thum	*1972*	Poplars	Unknown, possibly illegal import of propagation	Possibly inadequate quarantine interception
Melampsora larici-populina Kleb	*1973*	Poplars	Unknown, possibly illegal import of propagation	Possibly inadequate quarantine interception
Melampsora epitae Thum	*1972*	Willows	Unknown, possibly illegal import of propagation	Possibly inadequate quarantine interception
Melampsora coleosporioides Dietel	*1978*	Willows	Unknown, possibly illegal import of propagation	Possibly inadequate quarantine interception
Dothistroma septospora *(Dorog.) Morelet;[syn. D. pini Hulbary]; sexual stage: Scirrhia pini Funk & Parker.*	*1975*	Pines	Unknown, suspected by trans-Tasman air currents from New Zealand	Natural invasion from New Zealand
Phaeocryptopus gaeumannii (Rhode) Petr. [syn. *Adelopus gaeumannii* Rhode]	*1974*	Douglas fir	Unknown	Unknown
Marssonina castagnei (Desm. & Mont.) Magn.	*1984*	Poplars	Unknown, possibly illegal import of propagating material	Possibly inadequate quarantine interception
Marssonina brunnea *(Ell. & Ev.) Magn.*	*1987*	Poplars	Unknown, suspected by trans-Tasman air currents from New Zealand	Natural invasion from New Zealand

*(adapted from Old and Dudzinski. 1999)

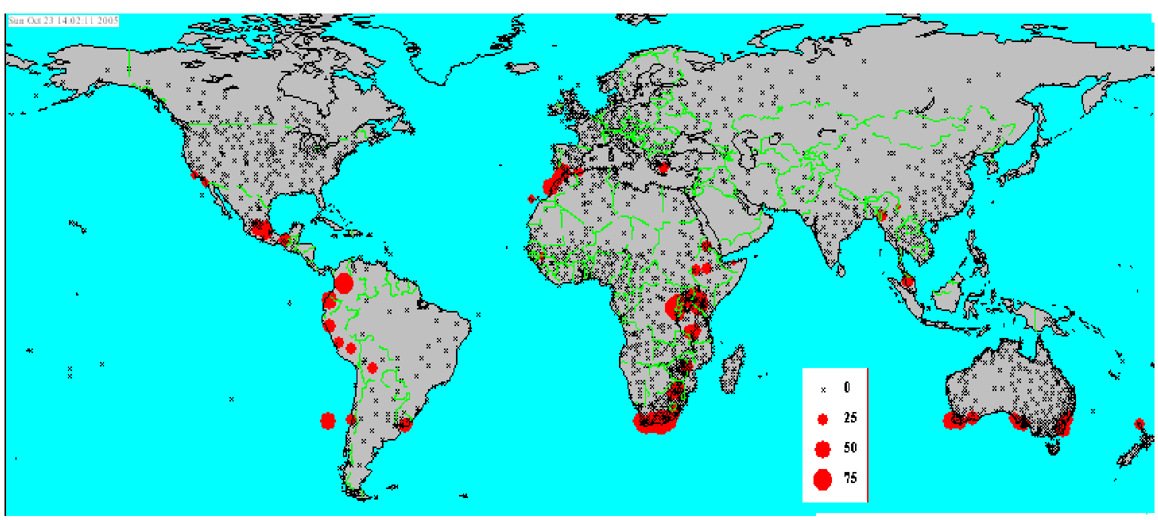
Disease and pathogen control is a daunting task and some would say futile. Often by the time the disease or pathogen is identified in a new area, it is already too late. One diseased or symptomatic tree could be managed if found and tested, but as there are over 4.5 million hectares (Balocchi et al., 1999), this seems an impossible feat. This daunting task of identification is only made easy once the pathogen or disease is at epidemic levels, where the damage is more easily identifiable. This trend is not dissimilar from history; in fact, when pitch canker was first discovered in Southern United States, it was not until it was at an epidemic level that it was noticed, at which time it was too late for control (as reported by Viljoen et al., 1997).

METHODS AND MATERIALS

Climex Model

One assumption that is fueling the investigation into potential resistant seed stock is that *Fusarium circinatum* could survive and pose a threat to exotic plantations of Monterey pine. A global perspective of this threat is shown in Figure 1. Using a Climex model climatic standards such as heat stress, cold stress, temperature, and moisture were altered to identify conditions which are necessary in both North and South America's populations of native *Pinus radiata.* After determining the appropriate parameters which allowed for *Pinus radiata* to exist, as close as possible, in known locations, this was then applied worldwide to determine other areas that have advantageous environments for *Pinus radiata.* Potentially, because of *Fusarium circinatum* success in native stands of *Pinus radiata*, this model could also represent potential habitat for *Fusarium circinatum.*

Figure 1. Climex model for *Pinus radiata* and its possible associate, *Fusarium circinatum*



(Key: x = comparison climatic data, otherwise, the red dots size is proportional to the expected success of the modeled species)
Note: The red dots do not represent a specific level, just a relative prediction, the larger the dot, the more likely the organism could succeed there.

An intriguing picture of the potential spread threat is depicted in Table 3 and Figure 3. Yes, California and the international Monterey pine dependent countries are represented in the model and are thusly similar, but are they close enough to predict an outbreak of *Fusarium circinatum*? It is hard to say, especially with evidence, that it has been found in nurseries in South Africa, Chile and Spain and did not establish itself in plantations. The best assumption is the more conservative view that the climates are similar enough to allow for *Fusarium circinatum* to spread into the commercial plantations abroad especially considering its potential for sexual reproduction.

The IMPACT Project

The project's incipient stages began from a suggestion by Bill Dyck to CSIRO (currently known as ENSIS) in response to the perceived immense threat of pitch canker as it occurs in the United States to infect the plantation dependent countries. The Impact project was designed at the 1998 IMPACT workshop at "Monterey". The conference was held at the Asilomar Conference Grounds in Pacific Grove, California. During this conference, committees and subcommittees were formed with the goal of identifying a nursery which could satisfy the needs for the two projects, as well as a site selection committee to select appropriate locations for a field trial. The first question as to what nursery was capable of propagating the some 14 or more thousand seedlings was answered by the selection of the IFG or Institute of Forest Genetics in Placerville, California, which is a branch within the USDA Forest Service. The IFG, incidentally, was the second choice behind the USDA Forest Services' nursery near Chico, California, but once it was decided that the two phases would be spaced apart by one or more years, the IFG was capable of that number of trees. Once that was decided, the other question of identifying a site for a field trial was determined when they selected a field at Swanton Pacific Ranch, a property of California Polytechnic State University in Davenport, California, known as the "Hay field." The original goal was two locations, but the other site which was to be a Big Creek Timber Company field, fell through.

With the nursery identified and the field location solidified, the project could go forward, but what exactly was to be done? There are two phases known as IMPACT phases I and II. Phase I was the artificial inoculation phase, where the seedlings which were propagated at an inoculums-free location were transported to another greenhouse which would not be adversely affected by the presence of pitch canker. At this location, the seedlings of known "stock code" (code associated with the various genetic stock provided by the participating countries) would be artificially inoculated with *Fusarium circinatum,* and they would record the size of the lesion which formed in response to the fungus. The results from this process are contained in the CSIRO Forestry and Forest Products Client Report 1283 and Southern Tree Breeding Association Technical Report TR02-07. 30pp. (unpublished) by Matheson et al., 2002. Once the seedlings were shipped off to Pebble Beach Company, the site for phase I, the IFG went to work propagating the rest of the seeds to establish the population for the field trial, known as phase II.

Phase II is the phase which is contained within this thesis project. Phase II was designed to be the phase where the various stock codes were out-planted in a field trial in the presence of "natural inoculums." The goal of this phase was to see how pitch canker spread through a stand without artificial inoculation. It is not the goal of this project to catalog the lesion lengths associated with infection, but rather to determine the natural movement/infection of the plantation. This phase will be terminated in 2011 prior to its 6^{th} anniversary planting. After this time the population would be reaching sexual maturity, and the possibility of interbreeding with the native population could occur.

This thesis is a portion of phase II; it is not at all the complete analysis. Rather, the goal as determined by Dr. Walter Mark of Cal Poly, San Luis Obispo, my mentor professor, and I, was to identify the sources of unequal survival among the seedlings independent and dependent on pitch canker. The in-depth investigation attempting to explain the inequalities among the various stock codes and their associated survival rates follows.

Hypothesis Statements

At a few points while setting up this project it appeared that there were a few areas that should be evaluated to determine the applicability of the results. These areas of interest became my hypothesis statements: (1) The stock's origin and sub-origin will affect their survival and establishment of the Monterey pine in the field trial. (2) The origin and sub-origin of the seedlings will respond differently to a natural infection of *Fusarium circinatum*. (3) Soil moisture or position in the drainage will impact survival. throughout the project's timeline. Other questions will form later, only in an attempt to answer these guiding statements.

Project/Plantation Design

Time zero (the planting date) for the plantation was the 12th of February 2005, but that is not when time was first spent on its design, Dr. Spencer (CSIRO) and Dr. Mark (Cal Poly, San Luis Obispo) devoted time developing the outline for the planting. They took into account the dimensions of the field, the number of available seed, and the future statistical validity of the project. The result was Figure 2.

Figure 2. Field Design

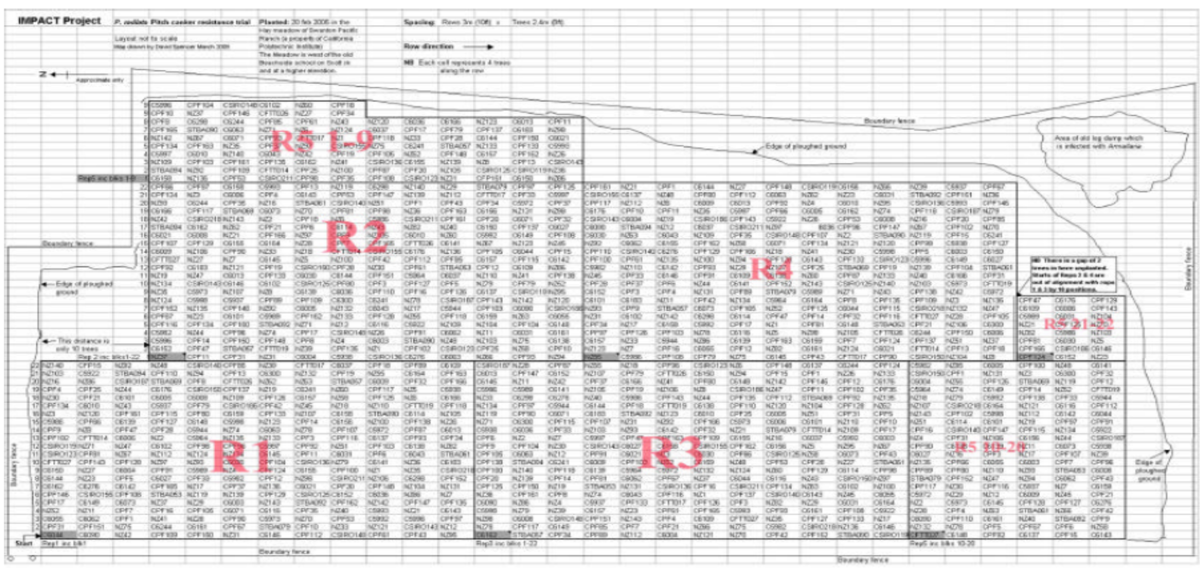


*Diagram comes from Spencer et al. 2005, client report no. 1581

A diagram of the plot design is presented in Figure 2. The design is that of a complete randomized block design. The overall area which is approximately 9 acres, contains 5 “blocks,” or as we titled them, replications. Rep 1, Rep 2, Rep 3 and Rep 4 are continuous; Rep 5 had to be “pieced” in order to fit seedlings in the area provided avoiding the possible bias if it were planted in the existing *Armillaria* spp pocket in the South east corner (or in the figure’s top right corner). The layout for each replication is 48 trees in the north-south orientation (rows), and there are 22 rows in the east-west orientation (columns) (See Appendix A for all of the replication layouts). Each of the columns is ten feet apart, and within each column the trees are planted with 8 foot spacings. The result is that across the entire plantation there is an 8 x 10 ft spacing between the trees. Further explanation of each column is required. Within each column, when looking at one of the sample replication layouts, there are 12 stock codes, each code represents 4 trees of the same codes. For example, on the replication chart it reads in column 1 C5992 , CPF34, NZ 112, there are four C5992, followed by four CPF34's and four NZ112's. Each of the stock codes which were represented in this trial is listed in Appendix B. Specifically there are 264 different stock codes represented four times per replication resulting in 1056 individual seedlings per replication. Under the layout description of a completely randomized block design, each of the stock codes (which represent 4 like trees) exists randomly in each of the replications. The block design is supposed to make up for the inevitable differences inherent in the soil properties, moisture content, slope and other variables that differ among the 5 blocks.

Description of Trial Design

Following the initial planting of the seedlings in the "hay field" (the informal title of the location on Swanton Pacific Ranch) the plan was to visit it twice per year to catalog its survival. The first visit was in November 2005. In that visit, the plantation's survival rates were cataloged. Noticing abnormally high level of mortality (in most of the replications, a 38% or higher mortality rate) and because the IFG still had many of the seedlings in the greenhouse, the decision was made to "re-plant" the gaps, or openings derived from mortality within the available seed stock. With continued support from the IFG, another planting was organized on February

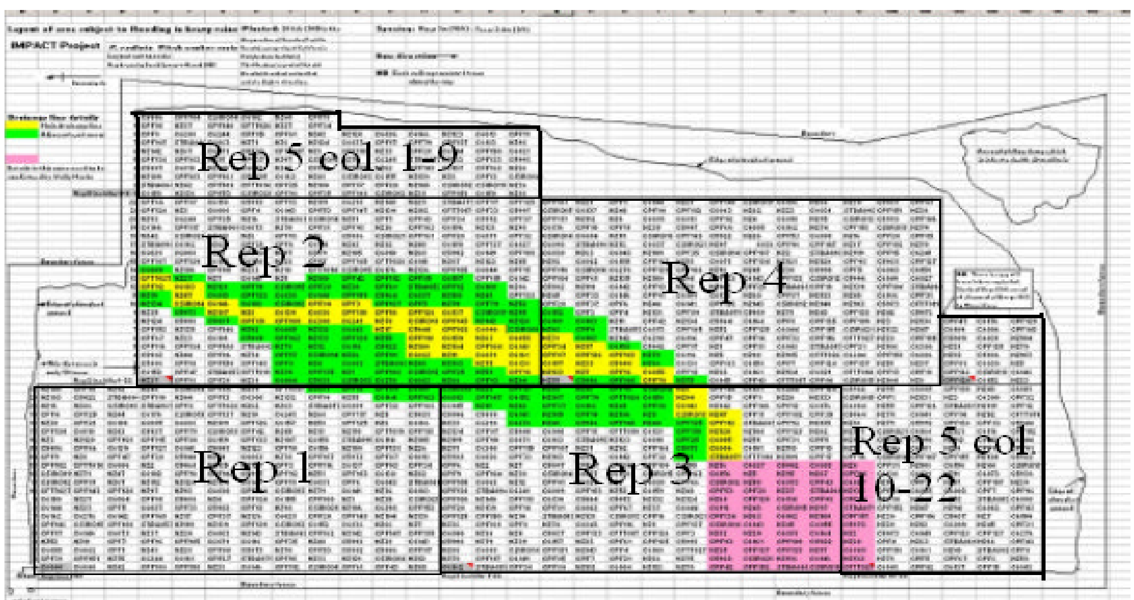
18, 2006. Once this “second planting,” otherwise represented as population 2, was established. It was surveyed again in April of 2006 to record the survival rates to have an “established” baseline for population 2 and an intermediary record of population 1’s 1.5 year survival rates. The third and final visit to the plantation in November of 2006 represented observation 3 for population 1 and observation 2 for population 2.

Considering the second planting of seedlings would have one year less of exposure to natural inoculums and other sources of mortality, a decision had to be made as to how to analyze the two populations. The initial assumption was that the second population could not be included as it would “skew” the results as it was not “fair” because that had one more year in the greenhouse and even though they were the identical stock planted at the same time, they could not be included as part of the overall population, and we would have to separate the data into two populations. One set of data being only population 1 data, following only the seedlings which were planted in February of 2005 to their third observation in November 2006. The other set of data being population 1 combined with population 2. The description of this data is more complex, it includes not only the data surrounding the initial population, but when the replanting occurred, the plants that were “implanted” into the gaps, then increased the number of individual seedlings of that stock code which were in the trial. The result was that the overall number of trees per stock code were no longer equal, because if many of one stock code died in year one, there were many more infill planted into their location resulting in a higher number of individuals planted (over time) than the stock code with better survival, with only one or two infill planted into the 1st population’s gaps. For this purpose, the analyses of the two populations will be represented by the title of “population one only” or “population one and two.”

Analysis of the Two Populations

In statistics the best “first step” is typically always to graph the data, to determine if there are any relationships that were indiscernible in the raw data. However, before we analyze the graphs a few things must be considered. Time zero for the plantation was after some very wet weather. With substantial standing water in the natural drainage, 1st year survival was impacted heavily by drainage patterns in the planting area. The drainage area is shown in Figure 3.

Figure 3. Plantation design with wet areas identified.



*adapted from David Spencer’s Diagram in Spencer et al., 2005

Figure 3 shows the area which was potentially impacted by an over abundance of water, the yellow areas showing the highest levels of water, the green slightly less, and the pink area proved to be of no concern. For analysis purposes, we will term the yellow area “C” for center of the drainage, the green area will be termed “E” for the edge, and the rest of the plantation will be described as “A” for average. The potential for soil moisture deficit as well as surplus to impact survival is high, so this must be investigated as a contributing factor to mortality.

Assuming that the block design satisfies or corrects the various inherent environmental differences between the replications, what else could need to be explained? When analyzing the data, reading, and discussing the spreadsheets provided by Annie Mix and Detlev R. Vogler from the USDA Forest Service’s Institute of Forest Genetics (IFG) in Placerville, California, we observed some noticeable differences in seed size, age, and proper storage techniques which were attributed to management differences between the involved countries or tree breeders. Rather than trying to classify the various differences, the decision was made to analyze data based on each of the stock code’s macro origin, as well as their micro origin or sub origin, which is their origin beyond simply Chile or Australia. This information regarding sub origin was accessible in the Chile and Australia stock, but not for the New Zealand stock. When investigating whether or not this would be acceptable, the data contained in Appendix E[1] which records the various stock codes’ seed germination rates was reviewed. From this review it was apparent that the New Zealand seed stock was more or less, of equal quality and vigor suggesting similar seed collection methods and quality standards. Considering this, the determination was made to include only one sub origin for the New Zealand stock.

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[1][1] *Annie Mix and Dr. Detlev Vogler from the Forest Service’s Institute of Forest Genetics recorded seed germination data relative to each of the stock codes. This data is contained in Appendix E.

So far the analysis will include soil moisture, origin and sub origin, replication membership (1, 2, 3, 4 or 5), and the overall survival percentages across all three observations for all of the stock. Tables 6 through 11 include in the analysis data which allows for the two population identities (planting date); population one and population one and two combined. The resulting SAS reports include the conservative as well as liberal tests results.

Table 6: Analysis Variables and Categories:

<u>**Origins:**</u>	<u>**Replication memberships**</u>**:**
1. Chile	1. Rep 1
2. Australia	2. Rep 2
3. New Zealand	3. Rep 3
<u>**Sub Origins**</u>**:**	4. Rep 4
1. Australia (C coded stock)	5. Rep 5
2. Bosques Arauco	
3. Forestal Celco	<u>**Wetness Categories**</u>**:**
4. Forestal Bio bio	1. Average (A)
5. Forestal Cholguan	2. Edge of Drainage area (E)
6. Forestal Millalemu	3. Center of the Drainage (C)
7. Forestal Mininco	
8. Tornagaleones	<u>**Observations**</u>**:**
9. Forestal Valdivia	1. Observation 1 (Nov, 2005)
10. Bosques de Chile	2. Observation 2 (Apr, 2006)
11. Cementos Bio bio	3. Observation 3 (Nov, 2006)
12. Australia (CSIRO)	
13. Australia (CFTT)	<u>**Lesion**</u>**:**
14. Australia (STBA)	1. Lesion length results from the “Glass house trial” or IMPACT I.
15. New Zealand (only)	

Graphs and Comparison Tables

The first step, once the parameters have been set, is to graph the data and look at the tables to see if there are any obvious relationships, or potential issues that could violate assumptions required for valid statistical analysis. Considering the various relationships to be analyzed, the first: Is there a relationship between survivorship and sub origin?

Figure 4. Relationship between survival and sub origin, population one only

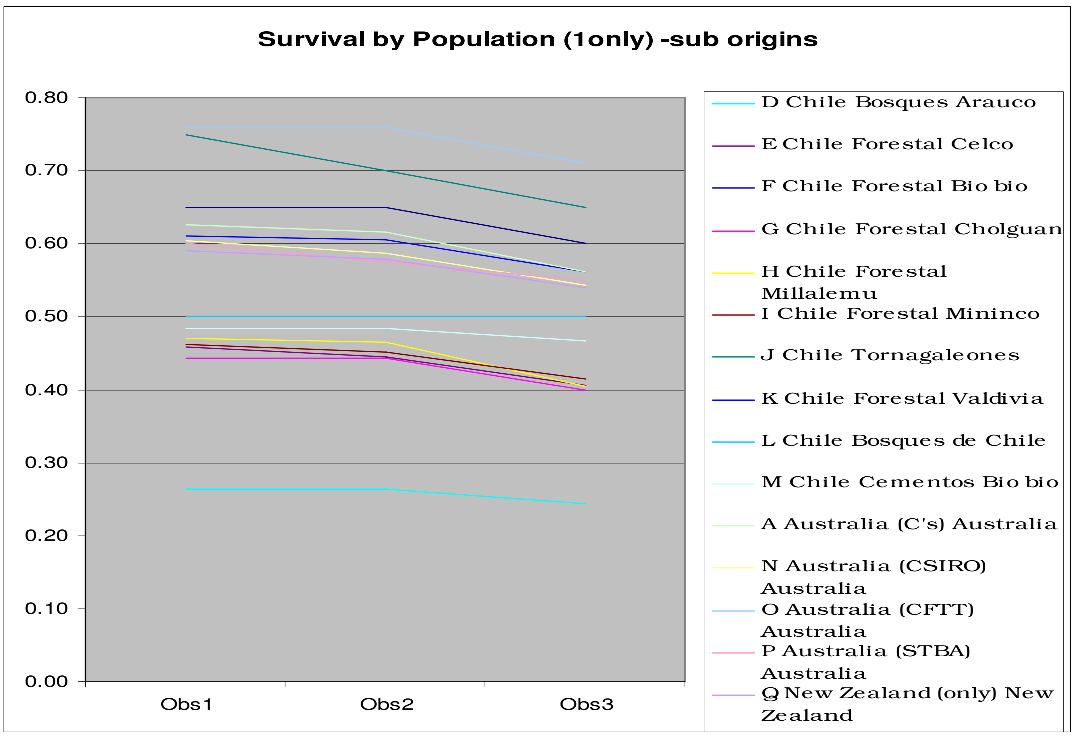
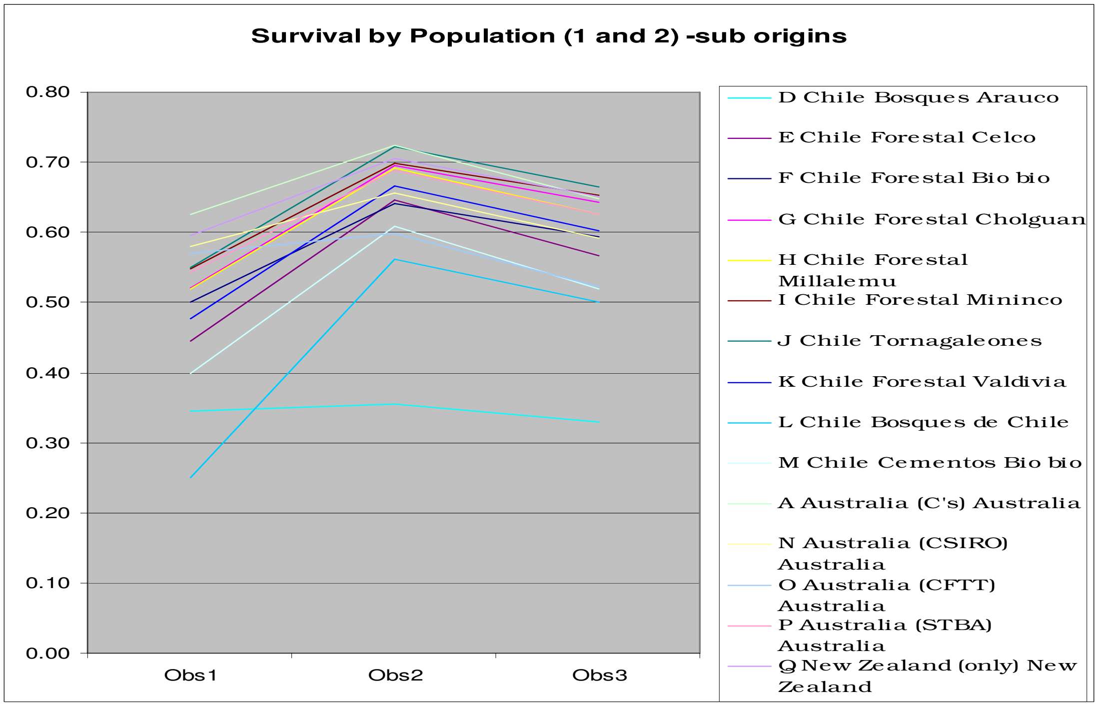


Figure 5. Relationship between survival and sub origin, population one and two



The appearance of the two graphs, Figure 4 including only the first population and Figure 5 including the initial population in addition to the replant have some similarities. Figure 4 shows an obviously deficient sub origin that vividly stands out, and that is sub origin category “D” or the Chilean tree breeding group known as Bosques Arauco. Figure 5, looking past the variable slopes from observation one to observation two related to the prevalence of seedling stock to be replanted, the ones with steeper upward slopes had more trees replanted than the lesser sloped sub origins, shared in the obvious deficiency within the “D” sub origin. The graphs do not show any characteristics regarding any possible violations of normality, independence and equal variance are the critical assumptions in the statistical analysis. An interesting observation can be made between observation one and two when reviewing Figure 4. Nearly all of the stock maintained their 1st survival rate through observation two, and then began a steady decline to the third observation. The potential implication of this observation is that, not until the seedlings were of greater size and given time to be infected by *Fusarium circinatum* did the plantation's survival rates begin to show obvious signs of "disease" activity. In other words, in many cases, certainly not all of the mortality between observation two and three, but much of the mortality has potentially been induced by *Fusarium circinatum*. The category of origin proved to display a similar relationship (Figures 6 and 7), with almost parallel slopes generally similar survival percentages across all three observations.

Figure 6. Survival by origin, population one and two

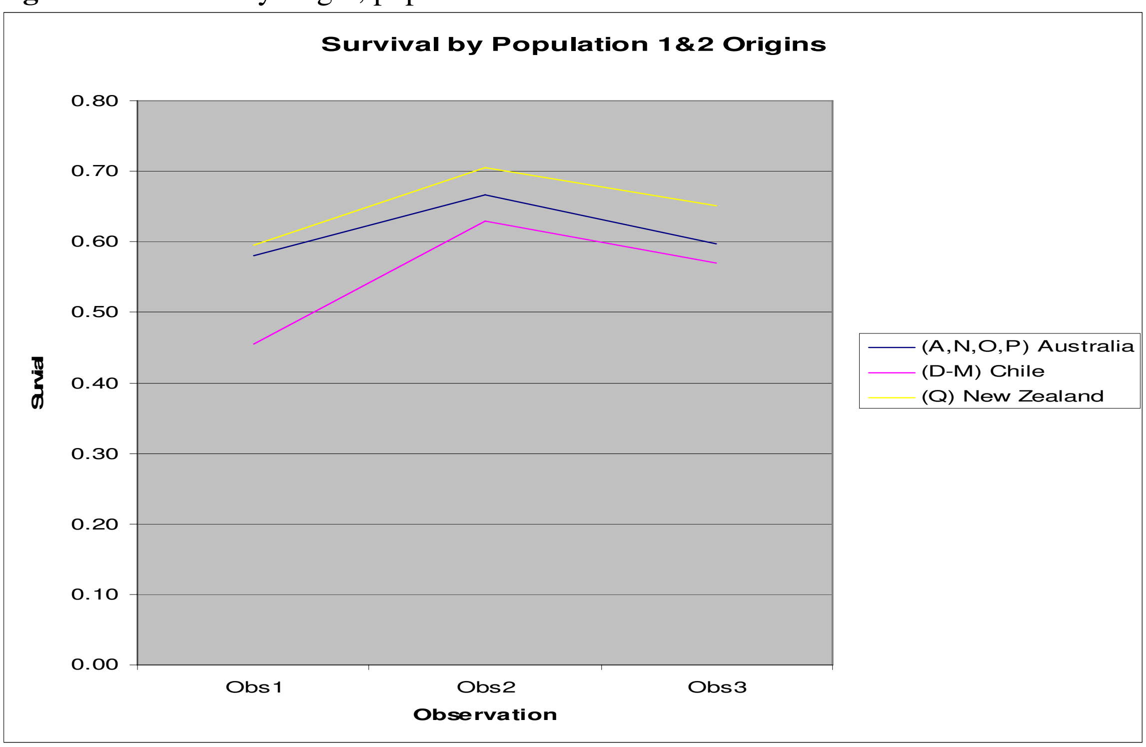
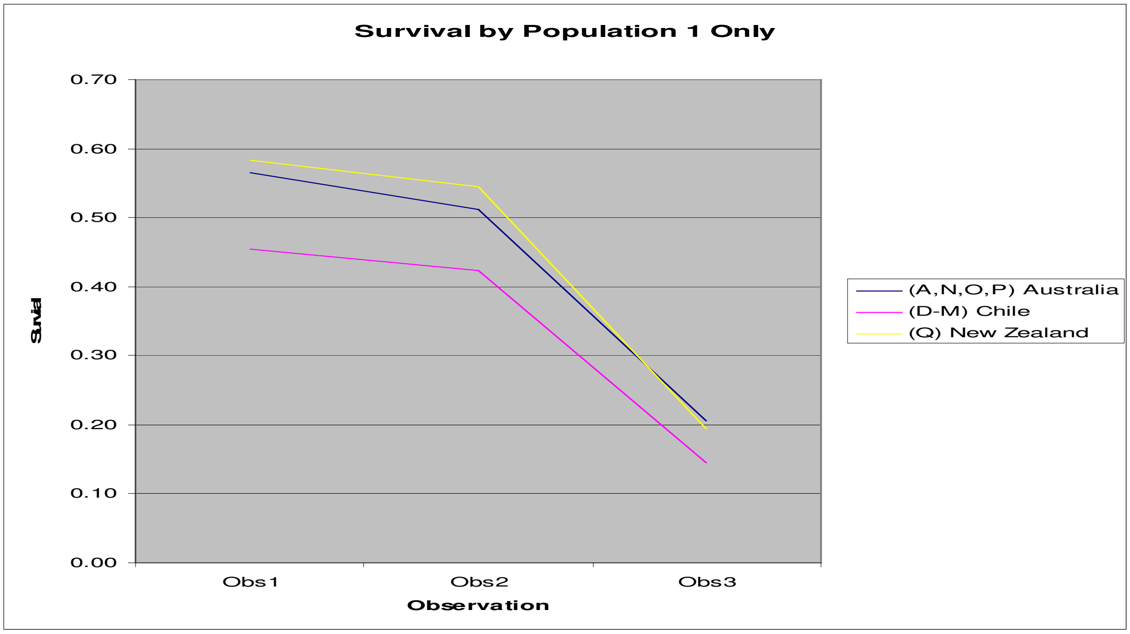


Figure 7. Survival by origin, population one only



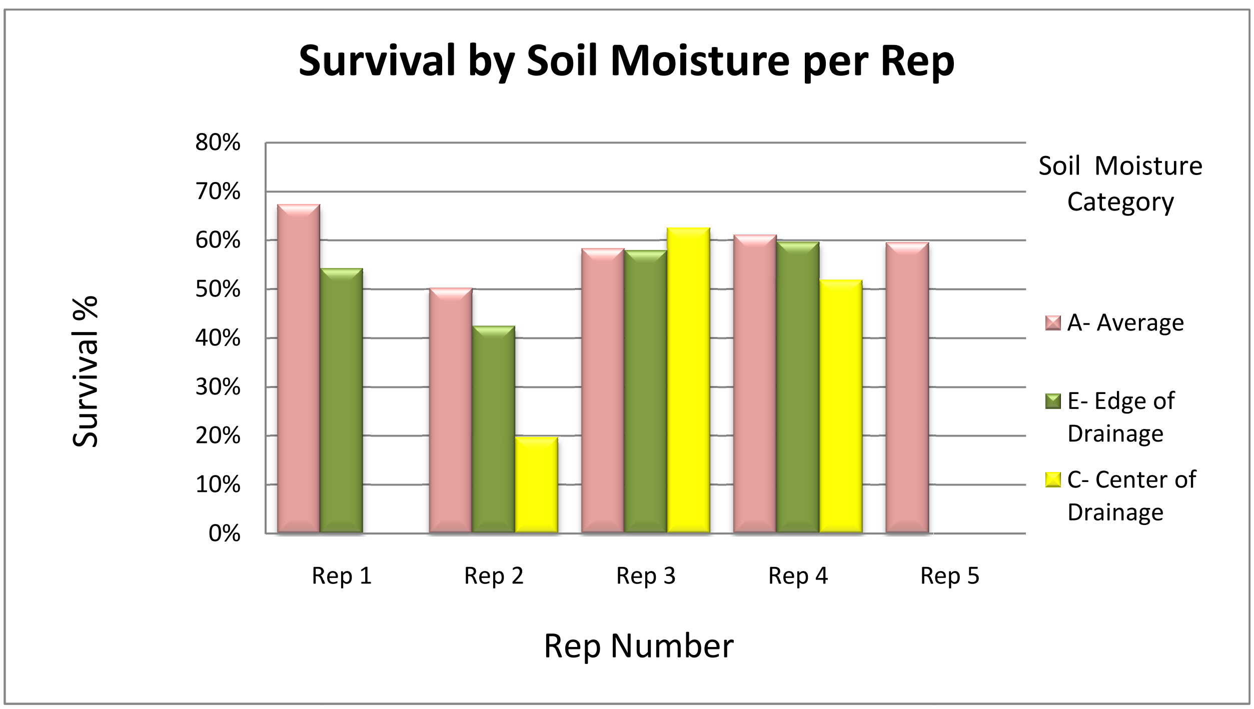
The initial analysis of Figure 6 and 7 supports the theory that the origins and sub origins of the stock may impact the survival of the individual seedlings. This would prove to be important in the final analysis, because if the simple origin of the seed stock has a significant impact on the seedling's survival, perhaps the experiment was not properly established. Ensuring that the seed was of similar quality was a critical assumption since three dissimilar populations cannot be compared.

Would this prove to be the demise of the experiment? No, the resulting conclusions would simply have to account for this. If the population's survival is impacted by seed quality or other variables included in the origin category, not necessarily the inherent resistance to *Fusarium circinatum*, then the data must be segregated relative to the population from which it originated. In other words, we should not expect the same survival percentages from the Chilean origin and the New Zealand origin, or the C stock from Australia and the Bosques de Chile in the population one only data.

Each of the origins depicts an average; each contains great performers and some very poor performers, if we only analyze the top 8 of each origin, the picture is quite different. The top 50 stock codes for New Zealand have 100% survival all of the way in the third observation, the top 32 for Chile and the top 74 from Australia also have 100% survival in observation number 3. See Appendix C.

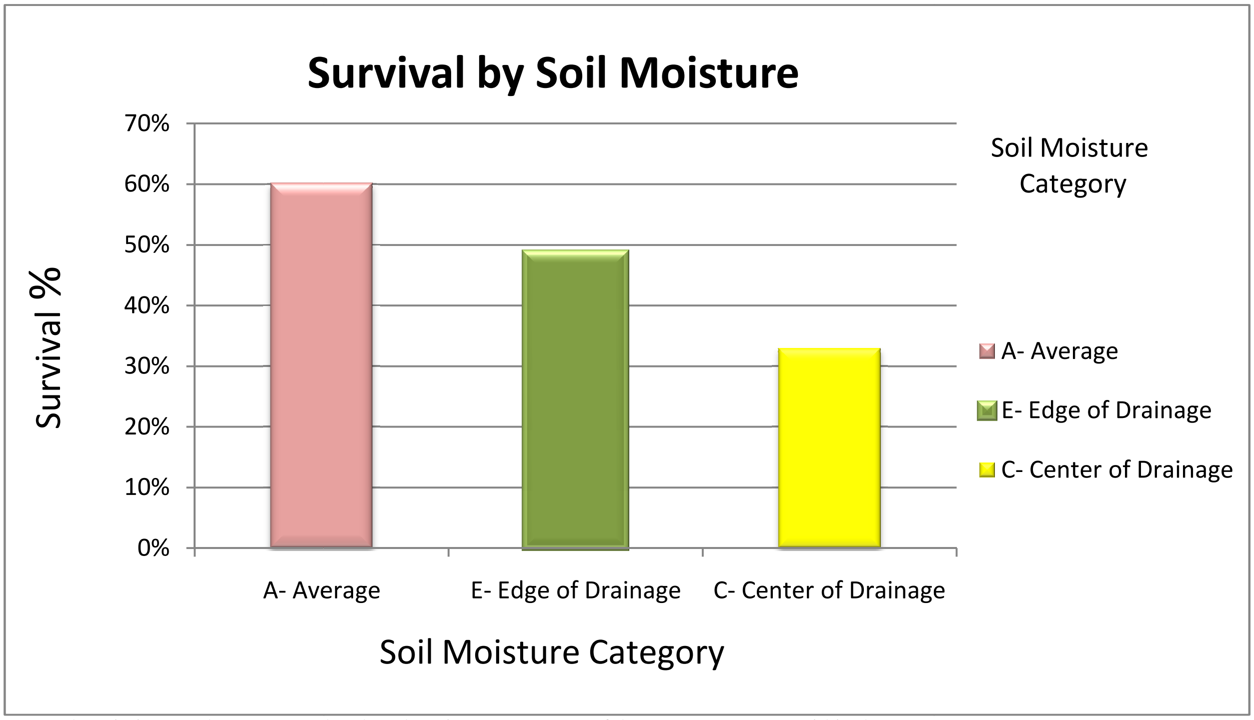
There seems to be a difference in survival by origin and sub origin, which implies that the seed which created the seedlings included some inherent restrictions on their potential for success, but what else could impact their survival? As was mentioned earlier, the soil moisture also has the potential to impact survival; the following graphs show the differences in survival rates in observation 3, by rep, and by soil moisture (derived from location in the drainage). See Appendix D for all raw data including wetness, lesion length, survival by observation, origin and sub origin.

Figure 8. Survival by wetness by rep



*Note, the missing rep data occurs only when there is no occurrence of the wetness category within that rep

Figure 9. Average Survival by wetness across all reps



*Note, the missing rep data occurs only when there is no occurrence of the wetness category within that rep

SUMMARY/CONCLUSIONS

Statistical Analysis

Table 7 shows the results from a multivariate repeated measures test in SAS which tested the hypothesis that soil moisture impacted the establishment of seedlings. The statistical analysis concludes that in both forms of analyses, both liberal and conservative, population one only, at time one, the soil moisture or "wetness column" was significant. In the analysis using both populations one and two, survival rates were significantly affected. Due to the limited degrees of freedom, it was not possible to determine soil moistures effect at time three (Observation 3). However in population one only, the soil moisture which was determined by its position in the drainage was only significant in the window of time between time zero and the first observation, from that time on it did not prove to be significant. The implication is that once the plants were established (after one spring and summer) in their respective locations, their position in the drainage did not affect their survival rates. For example if 70% of CSIRO's stock, due to random selection, was in the most saturated section of the drainage and did not perform well, this may not necessarily imply their lack of resistance. If there was stock available to replant into those openings however, based on this evaluation, after time 1 soil moisture was not an impacting variable and other factors including resistance to *Fusarium circinatum* could be affecting survivorship and thus the data collected is pertinent to management decisions.

Key to Interpreting the Results

The basics for interpreting all of the below results are as follows. Wilk's Lambda and Pillai's Trace are the most conservative tests, and Hotelling-Lawley Trace is followed by Roy's Greatest Root as the most liberal test for significance levels in Manova test criteria in F approximation. The derivative of their functions is the corresponding Pr>F value (Roy's greatest root this is an upper bound, for the rest it is the approximation), for most analyses if this value of alpha is less than .05 it is considered significant which refutes the null hypothesis. For example in both population one and two, the conservative/liberal tests yield the same conclusion, that there is a correlation between soil moisture and survival over time. The S value relates to the time category, in this case, S=1 is the correlation between moisture level and survival in time one, and S=2 being the correlation between moisture level and survival in time two. Of course at time 0 the survival rates for all of the seedlings regardless of location was 100%.

Table 7. Analysis of soil moisture's impact on survival

Population one only

Hypothesis: No correlation between soil moisture and survival in observation 1, 2, 3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
soil moisture	2	17.3280196	8.6640098	37.12	<.0001
Error	1317	307.426041	0.233429		

Population one only

Hypothesis: no time effect

S=1 M=0 N=657

Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.93969728	42.23	2	1316	<.0001
Pillai's Trace	0.06030272	42.23	2	1316	<.0001
Hotelling-Lawley Trace	0.06417250	42.23	2	1316	<.0001
Roy's Greatest Root	0.06417250	42.23	2	1316	<.0001

Population one only					
Hypothesis: no time*soil moisture effect					
S=2 M=0 N=657					
Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.99905275	0.31	4	2632	0.8702
Pillai's Trace	0.00094731	0.31	4	2634	0.8701
Hotelling-Lawley Trace	0.00094809	0.31	4	1578.2	0.8702
Roy's Greatest Root	0.00088223	0.58	2	1317	0.5595

Population one and two					
Hypothesis: No correlation between soil moisture and survival in observation 1, 2, 3					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
soil moisture	2	8.6900506	4.3450253	31.98	<.0001
Error	1317	178.93107	0.01358626		

Population one and two					
Hypothesis: no time effect					
S=1 M=0 N=657					
Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.70626261	273.66	2	1316	<.0001
Pillai's Trace	0.29373739	273.66	2	1316	<.0001
Hotelling-Lawley Trace	0.41590392	273.66	2	1316	<.0001
Roy's Greatest Root	0.41590392	273.66	2	1316	<.0001

Population one and two					
Hypothesis: no time effect					
S=2 M=-0.5 N=657					
Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.96478123	11.90	4	2632	<.0001
Pillai's Trace	0.03523703	11.81	4	2634	<.0001
Hotelling-Lawley Trace	0.03648549	12.00	4	1578.2	<.0001
Roy's Greatest Root	0.03595924	23.68	2	1317	<.0001

Table 8 evaluates lesion length as a predictor of survival in the field trial. The GLM multivariate repeated measures analysis was used to discover the relationship between the results from the glass house trial and the survival rates in this stage of the IMPACT project. The resulting PR value being less than .05 implies that lesion length is also related to survival; the shorter the mean lesion length, the great the probability of survivorship in the outplanting.

Table 8. Glass house lesion length correlation with field trial survivorship

Lesion Length Analysis
Population one and two
Lesion length correlation (Hypothesis: No relationship between lesion length and survival)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	4	6.65913717	1.66478429	13.50	<.0001
Lesion	181	31.0520044	0.17155804	1.39	0.0016
Error	779	96.0975628	0.01233601		

Lesion Length Analysis
Population one only
Lesion length correlation (Hypothesis: No relationship between lesion length and survival)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	4	13.3532471	3.3383118	15.44	<.0001
Lesion	181	55.9757961	0.3092585	1.43	0.0007
Error	779	168.408547	0.2161856		

Population 1 and 2				Population 1 only			
Sub Origin	Sub Origin Code	Mean Lesion Length	Mean survival (Obs 3)	Sub Origin	Sub Origin Code	Mean Lesion Length	Mean survival (Obs 3)
(C's) Australia	A	None	65%	(C's) Australia	A	None	56%
Bosques Arauco	D	26.54	63%	Bosques Arauco	D	26.54	48%
Forestal Celco	E	27.96	57%	Forestal Celco	E	27.96	41%
Forestal Bio bio	F	28.16	68%	Forestal Bio bio	F	28.16	60%
Forestal Cholguan	G	30.17	58%	Forestal Cholguan	G	30.17	40%
Forestal Millalemu	H	28.8	59%	Forestal Millalemu	H	28.8	41%
Forestal Mininco	I	27.96	60%	Forestal Mininco	I	27.96	41%
Tornagaleones	J	31.98	70%	Tornagaleones	J	31.98	65%
Forestal Valdivia	K	28.33	66%	Forestal Valdivia	K	28.33	56%
Bosques de Chile	L	24.82	63%	Bosques de Chile	L	24.82	50%
Cementos Bio bio	M	23.98	62%	Cementos Bio bio	M	23.98	47%
(CSIRO) Australia	N	27.06	58%	(CSIRO) Australia	N	27.06	54%
(CFTT) Australia	O	26.32	72%	(CFTT) Australia	O	26.32	71%
(STBA) Australia	P	25.82	58%	(STBA) Australia	P	25.82	55%
(only) New Zealand	Q	21.6	66%	(only) New Zealand	Q	21.6	54%

Table 9 evaluates the impact of origin and sub origin on seedling survival. The resulting output seems to imply that origin, even when nested as it is, in each of the stock, is significant, even when we break it down further, to sub origin, it too, is significant. Specifically, the test produced a significance level of .0028 which is certainly less than the alpha value of .05. While investigating this, we discovered that there is a time effect. To correct this we should continue on to a polynomial contrast for the stock effect. (Littell et al., 2002)

Table 9. Survival by rep, sub origin, stock (sub origin), origin, and stock (origin)

Population one only
Rep * Sub Origin * Stock(Sub Origin)

Repeated Measures Analysis of Variance
Hypothesis: There is no relationship between survival and the variables listed

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	4	21.9473903	5.4868476	26.55	<.0001
Origin	14	18.1732416	1.2980887	6.28	<.0001
stock(origin)	249	67.2347833	0.2700192	1.31	0.0028
Error	1052	217.3965838	0.2066500		

Hypothesis: no time effect
S=1 M=0 N=524.5

Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.93454941	36.8	2	1051	<.0001
Pillai's Trace	0.06545059	36.8	2	1051	<.0001
Hotelling-Lawley Trace	0.07003438	36.8	2	1051	<.0001
Roy's Greatest Root	0.07003438	36.8	2	1051	<.0001

Hypothesis: no time * rep effect
S=2 M=0.5 N=524.5

Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.98257651	2.32	2	2102	0.0178
Pillai's Trace	0.01743813	2.31	2	2104	0.0181
Hotelling-Lawley Trace	0.01771754	2.33	2	1499.1	0.0176
Roy's Greatest Root	0.01683200	4.43	2	1052	0.0015

Hypothesis: no time *sub origin effect
S=2 M=5.5 N=524.5

Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.98057073	0.74	28	2102	0.8357
Pillai's Trace	0.01951440	0.74	28	2104	0.8353
Hotelling-Lawley Trace	0.01972744	0.74	28	1850.3	0.8358
Roy's Greatest Root	0.01310061	0.98	14	1052	0.4670

Hypothesis: no time *stock(sub origin) effect
S=2 M=123 N=524.5

Statistic	Value	F-Value	Num DF	Den DF	Pr> F
Wilk's Lambda	0.64969801	1.02	498	2102	0.4068
Pillai's Trace	0.38784643	1.02	498	2104	0.4029
Hotelling-Lawley Trace	0.48138913	1.01	498	2079.4	0.4108
Roy's Greatest Root	0.25279224	1.07	249	1052	0.2467

Rep * Origin * Stock(Origin)
Repeated Measures Analysis of Variance
Hypothesis: There is no relationship between survival and the variables listed

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	4	21.9473903	5.4868476	26.55	<.0001
Origin	2	11.8490018	5.9245009	28.67	<.0001
stock(origin)	261	73.5590231	0.2818353	1.36	0.0005
Error	1052	217.396584	0.2066507		

Table 10 considers employs a polynomial contrast for the stock effect (Littell et al., 2002). When evaluating this section, the origin appears not to play a role in survival during time one or two. This result held true when sub origin was evaluated. However when sub origin was evaluated in the between subjects, it held significant. During time variable one and two, the Pr>F values were also insignificant. This finding was surprising. It was not what was not what was expected, so population one and two were tested in the same fashion to see if combining the two populations together would have the same results.

Table 10: Means separation analysis for origin

Means Separation Results
population one only
hypothesis: no relationship between survival and the variables listed

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	4	21.9473903	5.4868476	26.55	<.0001
Origin	2	11.8490018	5.9245009	28.67	<.0001
stock(origin)	261	73.5590231	0.2818353	1.36	0.0005
Error	1052	217.3965838	0.2066507		

Contrast Variable: Time 1					
Time_N represents the nth degree polynomial contrast for time					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	1.91093952	1.91093952	244.02	<.0001
Rep	4	0.00807116	0.00201779	0.26	0.9050
Origin	2	0.02756065	0.01378032	1.76	0.1726
stock(origin)	261	1.92281042	0.00736709	0.94	0.7260
Error	1052	8.23818444	0.00783097		

Contrast Variable: Time 2					
Time_N represents the nth degree polynomial contrast for time					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean	1	0.25816507	0.25816507	78.49	<.0001
Rep	4	0.03393990	0.00848498	2.58	0.0360
Origin	2	0.00349045	0.00174523	0.53	0.5884
stock(origin)	261	0.87708313	0.00336047	1.02	0.4054
Error	1052	3.46015276	0.00328912		

Table 11 shows the results from evaluating the effect of origin and sub origin when combining the two populations. These results are dissimilar from the results discussed in Table 10. When combining the two populations, both time variables had significance, not entirely across the board because when stock and origin were nested the resulting Pr> F value was insignificant at 0.2341. Aside from that however, the rest of the variables were significant at both times. This result held true for sub origin as well, the values yielded the same result with the only insignificant value occurring within the nested stock sub origin variable. The implication for this is complex; when viewing the data strictly as population one (Table 10), the population's origin did not have a significant time effect at time one or two, but when considering population one and two together (Table 11), the time effect was significant.

Table 11: Populations one and two included in origin evaluation

GLM Procedure: Repeated Measures Analysis of Variance Hypothesis: no relationship between subjects.					
Source	DF	Type III SS	Mean Square	F Value	Pr> F
rep	4	11.3408158	2.8352039	23.6	<.0001
Origin	2	3.7876562	1.8938281	15.77	<.0001
stock(origin)	261	46.1356293	0.1767649	1.47	<.0001
Error	1052	126.3570190	0.1201112		
Contrast Time Variable: Time 1					
Source	DF	Type III SS	Mean Square	F Value	Pr> F
Mean	1	2.53888217	2.53888217	165.46	<.0001
rep	4	0.80133586	0.20033397	13.06	<.0001
Origin	2	1.24678702	0.62339531	40.63	<.0001
stock(origin)	261	4.28909690	0.01643332	1.07	0.2341
Error	1052	16.14186474	0.01534398		
Contrast Time Variable: Time 2					
Source	DF	Type III SS	Mean Square	F Value	Pr> F
Mean	1	7.8187569	7.8187569	1265.95	<.0001
rep	4	0.38665098	0.09666274	15.65	<.0001
Origin	2	0.33591510	0.16795755	27.19	<.0001
stock(origin)	261	2.12770453	0.00815212	1.32	0.0017
Error	1052	6.49738202	0.00617622		

Conclusions, Recommendations, and Management Implications

This project covers the conception of Monterey pine or *Pinus radiata* in a foreign environment, all the way to a scientific trial being conducted to evaluate its resistance to a major biological threat which is *Fusarium circinatum*. Monterey pine, a species capable of rapid growth rates, and also an effective economic resource for many countries, has the potential to be impacted by a fungus known as *Fusarium circinatum* or pitch canker. This threat was recognized by some forest stewards with foresight, and they all joined together in collaboration to discuss the potential threat of pitch canker in 1998. The result of this collaboration between Chile, New Zealand and Australia was the creation and design of a project known as the IMPACT project. The project, with its multiple phases, covered the gamut of natural and artificial inoculation. This paper was written to discuss the results from a portion of the natural "field trial" setting to determine the survival statistics related to plantation seedlings in an environment where exposure to natural inoculums is present. The plantation will continue to grow, and may ultimately suffer some mortality from impacts due to pitch canker. Eventually, it will be cut down to prevent pollination and sexual breeding between the native population and the non-native plantation population in the Hay field at Swanton Pacific Ranch, Cal Poly, San Luis Obispo's Ranch in Davenport, California.

This portion of the field trial design was to attempt to explain some of the relationships and interactions that were causing mortality in the stand independent and dependent on *Fusarium circinatum*. We concluded that the moisture levels which were dependent on the plants' location in the drainage had a significant negative effect on survival during their establishment phase, but after that phase, the moisture levels were not significant. We concluded that lesion lengths provided by the Pebble Beach Company Glass House trial in IMPACT Phase I did correlate with survival in the plantation. A high average lesion for a stock does, in fact, mean that the plant is less likely to survive over time in the natural setting. One issue that arose from this discussion is how to solve the problem of impacted survival by soil moisture. It is an assumption (for the manager who will be implementing these results), because lesion length proved to have a significant role in the survival of the seedling in the "out-planting" in a natural forest setting and the seed stock was significantly impacted by soil moisture. With this assumption it would be recommended that the manager use the lesion length results from the glass house trial to estimate what the survival would have been independent of soil moisture and other environmental factors.

Beyond the simple explanation of soil moisture's impact on survival and lesion length's correlation with survival, we continued to probe into other sources of unequal survival rates. In part because of simple observation by this author and professor, Dr. Walter Mark, and discussion with the propagators, Annie Mix and DetLev Vogler, survivorship seemed to be impacted by origin and possibly even sub origin. It is true that all of the stock came at one time or another from the California or Mexican native populations, but over time with intensive breeding programs, the trees have changed to adapt to differing environmental conditions, and the simple difference between seed collection and storage quality also played a role in survival. The results showed that sub origin which was down to the individual tree farm, if the data was available, as well as the macro investigation of National origin (Chile, Australia, and New Zealand), had a significant role in survival, especially during the first year, or the establishment phase. Once established, though slightly hindered by origin, it did not prove to be as significant. When attempting a means separation, significance was only found when population one and two were combined. Even then it was only significant at the time variable 1.

What does the manager take home from this paper? If there is no concrete practical implication from this project, then it was simply a source of intellectual deliberation with no concrete value in the goal of protecting the vast plantations of Monterey pine. As a manager, it is the opinion of this author that you should begin by searching through the Appendices and identify the effect or role that soil moisture had on your stock code's survival, if it is great, then proceed to the next issue of lesion length, which at this point seems to be a significant part in the survival of the seedling. Meaning: that environmental conditions aside, the basic relationship between Monterey pine and fungal attack still has a significant role even during this young stage of the seedlings. For example when one reviews the raw data there are various survival rates within a stock across all replications. Some inequalities of survivorship can be explained by location in the drainage, some as deer damage, presumed damage by *Endocronartium harknessii* (Moore) Hirat, and *Dothistroma pini* Hulbary, but others sources are unknown at this stage of the project. The project location is surrounded by native *Pinus radiata* which has an abundance of *Fusarium circinatum* impacting its own survival; it would be foolish based on the results from this investigation to ignore the potential that another source is specifically *Fusarim circinatum*. Later research has confirmed that Fusarim circinatum has impacted their survival.

Specifically let's review NZ97 which had a mean lesion length of 13.1 and average moisture levels in all replications it still has survivorship ranging from 0%-75% in observation 3 with an average survivorship of 40%. This one example shows the necessity to look at the outplanting in the big picture. On average across the project, the data supports the assumption that there is a positive relationship between shorter lesion lengths (in response to artificial inoculation) and survival. However because there are other variables it is not the only indicator for success. This comparison exemplifies the need as the manager to look at each specific stock code's variables (See Appendix F for entire raw data set). Other variable could include seed vigor, lesion length, location in the drainage and survival ratings prior to implementing a management change based on the results. For example if I were responsible for determining implementation of these results for the NZ stock codes I would rather select NZ 142 which had a mean lesion length of 15.29. The short lesion length implies an inherently stronger resistance to and artificial inoculation of *Fusarim circinatum* combined with a 75% survival through Observation 3. Specifically, NZ142 is in the top 20 out of the 264 stock codes implying natural resistance and strong performance.

As time goes by we have noticed and confirmed a lot of damage has been caused by western gall rust which appears to have been contracted while at the nursery and did not show identifiable symptoms until much later. The IMPACT project is in its second phase. My participation in Phase 2 is limited; I am only evaluating the establishment survival rates. Further research must be done, and has been done to identify the source of each of the mortalities as the trees grow and as symptoms become apparent it will be easier to identify the specific cause of mortality. The next stage for the manager would be to look at Figure 4 and 5 to identify where his or her stock ranked on performance. For example Chile's Bosques Arauco's performance was drastically lower than any of the other stock codes. Knowing that all *Pinus radiata* can be traced back to a small number of native trees, it is not likely that they have poor stock, rather, their storage methods, seed collection methods or other variables have affected its performance. This implies that perhaps a uniform storage/transportation methodology should have been imposed to ensure equality (See

Appendix E for information regarding seed propagation, data provided by Annie Mix and DetLev Vogler of the IFG in Placerville, CA).

RECOMMENDATIONS

At the conclusion of my role in Phase 2 of the IMPACT project some issues arose that needed to be reported. If this project was to be done again or a similar project was to be initiated somewhere else, there were some variables that should be addressed prior to the outplanting phase. Specifically, regarding seed stock, with the various suborigins employing various methods for seed storage, collection, and selection protocol it is not very appropriate to compare their survival percentages to other suborigins. I would recommend that there be a uniform collection/storage/transportation procedure adopted prior to shipping the various seed stock to the greenhouse for propagation. While discussing best practices for improving our ability to infer direct relationships, our ability to infer a resistance relationship was further hampered by the overwhelming infestation of western gall rust on the seedlings that were imported to the site, likely due to the IFG's proximity to an infestation of gall rust. I would recommend using an alternate greenhouse location for propagation to remove this variable as well. Though this variable may or may not have affected the earlier glass house trial, it did affect survivorship in Phase 2.

To remove other environmental variables would also be recommended such as damage by deer. I would recommend that an additional expense be incurred in the establishment phase to erect deer fence around the plantation to reduce the possibility of deer browse damage. Another inevitable variable was soil moisture. This project determined that between the time of planting and observation 1 was the only time that soil moisture had a negative effect on survivorship. It was unfortunate that during the establishment time, there was heavy rain and standing water in the bottom of the drainage (Replication 2).

Some variables such as rainfall cannot necessarily be addressed in future outplantings but the other variables can and should be. Specifically, the need to erect deer fence and to adopt uniform protocol for the seed stock, will improve the ability for direct relationships to be inferred between survival and the stock's resistance to a natural inoculation of *Fusarim ciricinatum*.

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Appendix F

Entire Raw Data Set Available on CD attached to this Thesis in PDF Form.

The data identifies alive (A), alive but symptomatic (AS) or dead (D) across all three dates for data collection.

Remember observation 1 is for Population 1 only. Observation 2 includes the second observation of Population 1 and the first observation of Population 2. Similarly, observation 3 is the third observation for Population 1 and the second observation for Population 2.